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SEMIANNUAL REPORT

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BIO-GAS PRODUCTION FROM ALLIGATOR WEEDS

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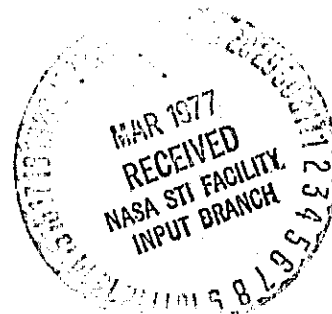
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Bio-gas Production From Alligator Weeds

Laboratory experiments were conducted to study the effect of temperature, sample preparation, reducing agents, light intensity and pH of the media, on bio-gas and methane production from the microbial anaerobic decomposition of alligator weeds (Alternanthera philoxeroides [Mart.] Griesb.). Efforts were also made for the isolation and characterization of the methanogenic bacteria, responsible for methane production. Briefly, methodology and results are presented as follows:

Materials and Methods

Alligator weeds were collected from a water cooling pond located at Crosby Chemical Company, Picayune, Mississippi. Harvesting was done manually by rakes and the plants were transported to the Alcorn State University Campus in plastic bags. Several fermentation units were constructed to conduct experiments to evaluate the effect of temperature, sample preparation, light intensity, reducing agents, and pH of the media, on the production of bio-gas and methane from the microbial anaerobic decomposition of alligator weeds.

A fermentation unit was constructed as follows: A large Coke bottle (970 ml) was sealed to the atmosphere with two hole rubber stopper. One outlet was fitted with a rubber septum for gas chromatographic sampling, and the other outlet was connected with plastic tubing to a sealed container filled with water acidified with sulfuric acid. The displacement of water in the second container by bio-gas produced in the fermentation bottle provided a convenient method of measuring the volume of bio-gas produced. Samples for gas chromatographic analysis were taken through the rubber septum. Perkins Elmer 811 Gas Chromatographs was used to estimate the methane content of the bio-gas.

Alligator weeds were chopped into approximately one inch long pieces and placed in fermentation bottles. Each fermentation bottle had 250 gram(wet weight) chopped plants, 20 ml of fresh rumen content from the cow's stomach and 250 ml of water. The purpose of adding inoculum from the rumen content was to initiate an aerobic decomposition-process and supply methanogenic bacterial populations. Since absolute anaerobic conditions are necessary for the growth of methanogenic bacteria, all units, once sealed, were not disturbed. A sealant was used to insure air-tightness around the tubes and rubber stopper. There were five treatments. Each treatment was replicated four times. The detail of various treatments is given below.

Treatment No. 1: Temperature

Four fermentation units were placed in an incubator maintained at 35°C and four other units were incubated at room temperature(24°C \pm 3°C).

Treatment No. 2: Sample Preparation

In one experiment plants were chopped into approximately one-inch long pieces. In the second, plants were blended into a slurry form and in the third experiment, the plants were first boiled in water for one hour and then blended into a slurry form.

Treatment No. 3: Light Intensity

In one experiment, four fermentation bottles were placed under bright light produced by fluorescent tubes(400 W). In the second, fermentation bottles were covered by aluminum foil to prevent exposure to light. In the third experiment, the fermentation bottles were left in the laboratory under ordinary light.

Treatment No. 4: Reducing Agents

Cysteine sulfide and cysteine hydrochloride were used as reducing agents. Cysteine sulfide was added to four fermentation bottles at the rate of 2 ml per 100 ml media whereas cysteine hydrochloride was added to the other four bottles at the same rate. The concentration of reducing agents was 2.5%. A control without the addition of reducing agents was also maintained.

Treatment No. 5: Effect of pH

The effect of pH was determined by adjusting the initial pH of liquid content in each fermentation bottle to 7, 8, and 9 respectively by the addition of sodium hydroxide. A control was maintained which had a pH of 6.6 .

All the experiments were conducted at room temperature ($24^{\circ}\text{C} \pm 3^{\circ}\text{C}$) with the exception of experiments of treatment number 1, in which the incubation temperature for one experiment was 35°C . In all the experiments plants were chopped into approximately one-inch long pieces with the exception of treatment number 2, where plants were blended into a slurry form with and without boiling the plants. Each fermentation bottle had 250 gram (wet weight) alligator weeds, 20 ml of fresh rumen content and 250 ml water.

Isolation and Characterization of Methanogenic Bacteria

Samples for bacterial isolation and characterization were taken from those fermentation units which had produced the highest amount of bio-gas and methane during anaerobic decomposition of alligator weeds. These units were those which were incubated at 35°C and those in which the initial pH of the liquid media was adjusted to 9. During the process of isolating pure cultures from these anaerobic microorganisms, strict anaerobic conditions were maintained as described by Bryant et al. The process of isolation and characterization is still in progress. The result of this study will be reported in the annual report which will be submitted in May 1977.

Results:

The data from the various experiments which were conducted to study the effect of temperature, sample preparation, reducing agents, light intensity and pH of the media, on bio-gas and methane production from the microbial anaerobic decomposition of alligator weeds, are presented in Tables 1, 2, 3, 4, and 5 respectively.

Temperature

Effect of temperature on the rate and amount of bio-gas production as well as on the percentage of methane in the bio-gas was very prominent (Table 1).

Increasing incubation temperature of the fermentation units from $24^{\circ}\text{C} \pm 3^{\circ}\text{C}$ to 35°C increased not only the rate of bio-gas production but also the methane percentage in the bio-gas. The methane content of total bio-gas was increased from 58.1% to 66.0% by increasing the incubation temperature to 35°C . Further more higher temperature also decreased the lag time between the production of bio-gas and production of methane gas.

Sample Preparation

Data presented in Table 2, revealed that sample preparation, (alligator weeds chopped, blended, and boiled and blended) had no effect on the total bio-gas production. However the methane production from samples which were boiled and blended, was reduced to a great extent (47.7%) when compared with chopped or blended samples (58.1% and 62.1%).

Light Intensity

Comparison of the data from experiments in which fermentation units were exposed to different light intensity, in Table 3 revealed that total bio-gas produced under bright light was slightly higher when compared with ordinary light or dark conditions. On the other hand, methane content of the bio-gas

was more (66.61) when bio-gas was produced under darkness.

Reducing Agents

According to the data presented in Table 4, addition of cysteine sulfide and cysteine hydrochloride to the fermentation units had slightly increased both, the bio-gas production and percentage of methane in the bio-gas. Cysteine sulfide was slightly more effect than cysteine hydrochloride.

Effect of pH

Data from the experiments in which pH of the liquid media in each fermentation unit was adjusted before anaerobic microbial decomposition of the alligator weeds, are presented in Table 5. The data showed that increasing pH of the media from 6.6 (control) to 7.0 had a slight decrease in bio-gas production as well as in the methane content of the bio-gas produced. However when the pH of the media was increased to 8.0, there was a little effect on bio-gas production but methane content of the bio-gas produced was increased up to 64.0%. By further increase in the pH of the media to 9.0, there was a significant increase in the bio-gas production as well as in the methane content of the bio-gas produced.

Table 1: Bio-gas and Methane Production From the Anaerobic Decomposition
of Alligator Weeds at Various Temperatures

No. Days Elapsed	Incubation Temperature			
	<u>24°C \pm 3°C</u>		<u>35°C</u>	
	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)
5	0	0	20	9
12	45	0	298	168
20	353	15	1180	613
42	940	503	1495	997
60	1351	788	1900	1248
81	2075	1363	2750	1768
<u>102</u>	<u>2726</u>	<u>1584</u>	<u>3195</u>	<u>2110</u>

Calculated Data:

% Methane
in Total Bio-gas

58.1

66.0

ml Bio-gas
Per Gram
Wet Weight

10.9

12.8

ml Methane
Per Gram
Wet Weight

6.3

8.4

Table 2: Effect of Sample Preparation on Bio-gas and Methane Production
From the Anaerobic Decomposition of Alligator Weeds

No. Days Elapsed	<u>Chopped</u>		<u>Blended</u>		<u>Boiled & Blended</u>	
	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)
5	0	0	0	0	0	0
12	45	0	27	0	59	0
20	353	15	307	39	429	78
42	940	503	885	426	1141	478
60	1351	788	1615	896	1739	750
81	2075	1363	2015	1196	2344	1064
<u>102</u>	<u>2726</u>	<u>1584</u>	<u>2603</u>	<u>1616</u>	<u>2654</u>	<u>1266</u>

Calculated Data:

% Methane in Total Bio-gas	58.1	62.1	47.7
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ml Bio-gas Per Gram Wet Weight	10.9	10.4	10.6
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ml Methane Per Gram Wet Weight	<u>6.3</u>	<u>6.5</u>	<u>5.1</u>
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Table 3. Bio-gas and Methane Production From the Anaerobic Decomposition
of Alligator Weeds Under Different Light Intensities

No. Days Elapsed	<u>Bright Light</u>		<u>Ordinary Lab Light</u>		<u>Dark</u>	
	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)
5	0	0	0	0	0	0
12	39	0	45	0	27	0
20	330	17	353	15	357	24
42	968	467	940	503	1067	529
60	1390	789	1351	788	1377	794
81	2155	1287	2075	1363	2256	1378
102	2840	1797	2726	1584	2678	1784

Calculated Data:

% Methane
in Total Bio-gas

63.3

58.1

66.6

ml Bio-gas
Per Gram
Wet Weight

11.4

10.9

10.7

ml Methane
Per Gram
Wet Weight

7.2

6.3

7.1

Table 4: Effect of Reducing Agents on Bio-gas and Methane Production

From the Anaerobic Decomposition of Alligator Weeds

No. Days Elapsed	<u>Cysteine Sulfide</u>		<u>Cysteine Hydrochloride</u>		<u>Control</u>	
	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)
5	0	0	0	0	0	0
12	70	24	50	17	45	0
20	584	364	470	307	353	15
42	1315	874	1145	729	940	503
60	1995	1264	1750	1124	1351	788
81	2595	1614	2420	1576	2075	1363
<u>102</u>	<u>2945</u>	<u>1804</u>	<u>2870</u>	<u>1706</u>	<u>2726</u>	<u>1584</u>

Calculated Data:

% Methane in Total Bio-gas	61.3	59.4	58.1
ml Bio-gas Per Gram Wet Weight	11.8	11.5	10.9
ml Methane Per Gram Wet Weight	<u>7.2</u>	<u>6.8</u>	<u>6.3</u>

Table 5: Bio-gas and Methane Production From the Anaerobic Decomposition
of Alligator Weeds at Various pH

No. Days Elapsed	Control(pH 6.6)		pH 7		pH 8		pH 9	
	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas (ml)	Cum. Methane (ml)
5	0	0	0	0	0	0	0	0
12	45	0	44	0	35	10	80	37
20	353	15	350	25	425	230	634	447
42	940	503	972	443	1133	730	1245	852
60	1351	788	1300	715	1242	805	1790	1385
81	2075	1363	1800	1065	1660	1107	2490	1822
102	2726	1584	2554	1475	2535	1622	2888	1964

Calculated Data:

% Methane in Total Bio-gas	58.1	57.8	64.0	68.0
ml Bio-gas Per Gram Wet Weight	10.9	10.2	10.1	11.5
ml Methane Per Gram Wet Weight	6.3	5.9	6.5	7.8